Terminology and Preparation Techniques of the Female Genitalia of Aedine Mosquitoes (Diptera: Culicidae)[1]

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ABSTRACT. Detailed procedures for preparing and dissecting the female genitalia of aedine mosquitoes for taxonomic examination are presented. A taxonomic glossary of the female genitalia of mosquitoes is given and the structures are illustrated.

INTRODUCTION

The female genitalia of aedine mosquitoes have been completely neglected or only superficially treated in most taxonomic studies even though they exhibit good characters for the separation of taxa. Neglect in the past of these structures by taxonomists probably resulted in part from the lack of specialized diagnostic preparation and dissecting techniques and the absence of a standardized descriptive terminology. Too often, earlier workers had only cursorily examined uncleared and/or improperly dissected genitalia and could not find any noticeable or consistent differences between species. Many previous studies of the female genitalia were also usually confined to examining the projecting external structures and comparing them at the species level. Unfortunately, the female genitalia usually possess few, if any, features (except subgenus *Neomacleaya* Theobald of *Aedes*) with which closely related species can be separated; however, the genitalia exhibit good characters for distinguishing species groups, subgenera and genera.

During the course of several years work studying the taxonomic significance of the female genitalia of aedine mosquitoes, the following detailed method of preparing permanent slide mounts of these structures was devised. These procedures, though originally developed for use with aedine genera

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(members of tribe Aedini as outlined by Belkin, 1962), work equally well when used with the other genera of Culicidae. A list of female genitalia terms (including synonymous names) and a composite diagrammatic illustration of the taxonomically important structures is also presented (Figure 1). It is hoped that other investigators of mosquito systematics will critically examine, describe and illustrate the female genitalia of their respective groups and that the following preparation techniques and terminology will be useful to them.

Terminology of the female genitalia presented here is adapted and modified from those of Reinert (1973) and Laffoon and Knight (1971). Other important papers with contributions to the taxonomy and/or terminology of aedine female genitalia are: Brolemann, 1919; Macfie and Ingram, 1922; Christophers, 1923; Barraud, 1928; Gerry, 1932: Gjullin, 1937; Edwards, 1941; Laffoon, 1946; Ross, 1947; Coher, 1948; La Casse and Yamaguti, 1950; Hara, 1947; Mattingly, 1958, 1959, 1961, 1970; Spielman, 1964; Delfinado, 1967, 1968; Mohrig, 1967, 1969; Rjazantseva, 1972; Zavortink, 1972; and Arnell, 1973. The female genitalia of all the subgenera of *Aedes* will be illustrated, completely described and compared to related taxa in a forthcoming publication.

PREPARATION PROCEDURE

The technique used to prepare a dissected, permanent, slide-mounted, female genitalia specimen consists of 14 consecutive steps as follows: (1) observe intact genitalia of pinned adult using a dissecting binocular microscope for the following: presence of undamaged external parts and preliminary observation of the presence and number of scales on the cerci, tergum VIII and sternum VIII; (2) place specimen in a relaxing jar[1], leave overnight if water is at room temperature or for 1-1 1/2 hours if water is hot (approximately 81-82°C); (3) remove adult from relaxing jar and attach a small label containing preparation number[2] to pin on which adult is mounted; a duplicate label should be kept with the clipped genitalia at all times throughout the preparation procedure and affixed on the final microscope slide mount; a record book containing the preparation numbers, collection data, identification, person making preparation and date of preparation should be kept for each genitalia prepared; (4) secure pinned adult to pinning block, clip abdomen between segments VI and VII with a fine pointed dissecting scissors, dissection should be performed while observing the specimen through a binocular dissecting microscope (10x ocular and 2x objective) which has a sheet of lens paper over its stage (lens paper allows for easier finding of accidentally

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[1]Relaxing jar consists of a large, wide-mouthed, glass jar with a tight fitting cover and an internal, removable glass or porcelain platform (containing numerous small holes) situated above the water line. Jar is filled to a level below platform with distilled water. Change water in jar every 3-4 days or add a small amount of carbolic acid to water to prevent mold growth.

[2]Preparation numbers can be made by using the prefix capital "T" (signifying terminalia), adding last 2 digits of current year and a decimal point (T74. for 1974) and finally adding consecutive arabic numbers for each preparation (T74.1, T74.2, etc.).

dropped genitalia); (5) transfer clipped terminal abdominal segments with genitalia to a 10 ml test tube 1/3 full of 10% potassium hydroxide (KOH) solution, which has been preheated to a temperature of 81-82°C in a water bath[3], return test tube containing specimen to water bath (81-82°C), leave for 10-15 minutes; (6) transfer specimen to a small watch glass (U. S. Bureau of Plant Industry model, 20 mm inside diameter) containing 5% acetic acid and 2-3 drops of stock acid fusion stain[4], leave for 6-10 minutes; (7) transfer specimen to a 10 ml test tube 1/3 full of liquid phenol containing a light acid fuchsin stain, leave for 1 hour; (8) transfer specimen to a small drop of copal[5] on a microscope slide (a number 3 insect pin stuck in the end of a small diameter wooden applicator stick can be used to transfer a small drop of copal from a dark glass storage bottle to the slide), take exact measurements of retraction of cerci and tergum VIII into segment VII, record the absence or the extent of scaling on the cerci, tergum VIII and sternum VIII; (9) dissect specimen with the aid of a dissecting binocular microscope, using procedure outlined below (copal diluted with liquid phenol to a medium to thin viscosity is used instead of Canada balsam because of the ease with which prolonged dissections can be performed without the rapid forming of a thin dry film over its surface which occurs in Canada balsam and results in a "stringing" effect when the dissecting needles are removed); (10) transfer dissected genitalia parts and abdominal segments to a small, thin-layered drop of copal on the center of a glass microscope slide (1 x 3 inches) on the ends of which slide labels are affixed; I record on the left hand label the collection data from the adult and the genitalia preparation number along the lower margin and on the right hand label the identification (genus and species) of the specimen and the letters C-CB (signifying the mounting media used--copal and Canada balsam) along the lower margin; (11) place slide in dust free slide drying oven which is preheated to a temperature of 45°C, leave overnight; (12) remove slide from oven, put 2-3 drops of moderately thin Canada balsam over the specimen in copal and cover with a 15 mm (number 1 thickness) circular glass cover slip; (13) return slide to slide drying oven (45°C) for approximately 24 hours at the end of which time it can be removed and final measurements and observations made on the genitalia parts; and (14) return slide to slide drying oven (45°C) for approximately 3-4 weeks at the end of which time it can be stored in a horizontal position (specimen side up) in a microscope slide storage box.

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[3]Water bath can be made by using a 100 ml beaker containing a single layer of small glass beads on the bottom, filled 1/2 full of water and heated over a small laboratory hot plate.

[4]Stock acid fuchsin stain prepared by adding 0.5 gram of finely ground acid fuchsin to 25 ml of 10% acetic acid and 200 ml of distilled water, agitate, let stand until stain particles are dissolved, agitate and filter.

[5]Copal consists of high grade, pulverized, crystalline copal gum dissolved in liquid phenol and filtered with a vacuum pump system. Copal gum can be purchased from Edward Gurr Ltd., London, SW 14, England.

DISSECTION PROCEDURE

The technique of dissecting the female genitalia, mentioned in step 9 above, is composed of 6 steps arranged in sequence as follows: (1) extend segment VIII from segment VII and remove latter by tearing membrance between them with fine dissecting needles[6]; tease out debris by alternately gently applying and relaxing pressure on segment VIII with a dissecting needle; (2) transfer genitalia (with segment VIII and seminal capsules attached) and segment VII to 2 small, thin drops of copal (drops slightly to left of slide center) on a new, cleaned, microscope slide (step 10 of preparation procedure above); position segment VIII and genitalia lateral surface up, remove tergum VIII by carefully tearing intersegmental membrance around entire tergum starting at both sides of base and progressing toward apex which is freed last (as parts are removed by dissection, place them to the side of the drop of copal so they will not be damaged or be in the way when additional dissections are made); (3) rotate genitalia so that the outer surface of sternum VIII is down, tease seminal capsules from inside sternum VIII and cut spermathecal ducts; (4) return genitalia to lateral position, remove tergum IX by carefully tearing connecting membrane starting at basolateral margins and progress toward apex which is removed last being very careful not to damage bases of the cerci and prevent tearing of the median area of tergum IX; (5) rotate genitalia so that outer surface of sternum VIII is down, carefully tear apical intersegmental fold (which is often folded cephalad) starting at the lateral margins and work medially, tear should be along apical margin of sternum VIII and leaving the apical intersegmental fold attached to the lower vaginal lip and insula; (6) position each dissected part of the genitalia and abdominal segments in the following manner: abdominal segment VII with tergum up placed at lower margin of near copal drop; make a short extension of the far copal drop to the lower right, on this extension at the extreme right arrange tergum VIII (dorsal side up and apex pointing to far side of slide) in a completely flattened position, to the left of tergum VIII place sternum VIII (ventral side up and apex pointing to far side of slide) in a completely flattened position; make a short extension of copal to the right directly above the previous one, on it arrange tergum IX (dorsal side up and apex pointing to far side of slide) in a completely flattened position; the seminal capsules are arranged next to each other (lateral surfaces up) at the base of the second extension of copal; a third short extension of copal is made to the right from the upper margin of the copal drop, on its end the remaining piece of the genitalia is placed (dorsal surface of cerci down with apices pointing to far side of slide), the postgenital lobe lays in a flattened position dorsally to the basal portions of the cerci, the cerci are arranged in a completely flattened position (care should be taken not to destroy the peri-anal membrane); next the apex of the lower vaginal lip is carefully separated from the upper vaginal lip until both the upper and lower vaginal lips are completely opened and extended in a flattened position (extreme care should be taken not to tear the vaginal lips, insula or vaginal sclerites),

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[6]Dissecting needle constructed by pushing the base of a stainless steel, minuten insect pin into the end of a small diameter, wooden applicator stick with a pliers.

when the vaginal lips are arranged in a flattened position the vaginal sclerites (when present) are also in a flattened position; finally the insula with the attached apical intersegmental fold (removed from sternum VIII) are fully extended (but not removed) and arranged in a flattened position (usually the insula, in species with a lip-like one, is automatically in a flattened position when the lower vaginal lip is extended and flattened). The preparation next is labeled and continues with step 11 of the preparation procedure stated above.

TAXONOMIC GLOSSARY OF FEMALE GENITALIA

Accessory Gland Duct (AGDu) = A duct between the accessory gland duct aperture and the accessory gland, basal area (AGDuB) near aperture usually pigmented. Syn.: spermathecal gland duct base (several authors); caecal duct (Mattingly, 1957).

Apical Intersegmental Fold = Intersegmental membrane forming a fold, usually directed cephalad, situated between apical margin of sternum VIII and lower vaginal lip, spiculate, occasionally moderately to heavily pigmented, with insula on median apical area. Syn.: pre-atrial fold (Christophers, 1923).

Basolateral Seta (BlS) = A small, paired seta situated on the basal lateral area of tergum VIII and/or sternum VIII, may be present or absent, occasionally replaced by a small pale bulla; presumably sensory and may function in monitoring the degree of retraction of segment VIII.

Cercus (Ce) = A pair of conspicuous dorsal lobes posterior to tergum IX, with setae of varied development and number, spiculate, with or without scales, and sternobasal area attached to peri-anal membrane.

Cercus/Dorsal PGL Index = Dorsal cercus length divided by dorsal postgenital lobe length.

Cercus Index = Dorsal measurement of distance from apex to the most anterior point of base (dorsal cercus length) divided by measurement of width at 0.5 distance from apex (cercus width).

Dorsal Postgenital Lobe Index (Dorsal PGL Index) = Dorsal measurement of distance from apex to midpoint of peri-anal membrance attachment (dorsal PGL length) divided by measurement of width at 0.5 length (dorsal PGL width).

Hinge (H) = Point of articulation between the upper and lower vaginal lips. Syn.: angle of sigma (Christophers, 1923).

Insula (I) = A small plate, varied in development, attached to median area of the lower vaginal lip, spiculate, maybe tongue-like or lip-like, usually with setae or tuberculi. Syn.: ilot pilifere (Brolemann, 1919); insula plate (Gerry, 1932).

Lower Vaginal Lip (LVL) = Sclerotized and pigmented rim of the floor of the vagina, cephalad area articulates with upper vaginal lip at hinge, median caudal area joined to insula, usually spiculate. Syn.: bride ventrale (Brolemann, 1919); sigma (in part) (Christophers, 1923); sigma (Gerry, 1932); anterior lip of atrium (Barraud, 1928); stigmata (*Culiseta*) (Rees and Onishi, 1951); ventral arc of sigma (Snodgrass, 1959); preatrial sclerite (Laffoon, 1946); ventral lip (Curtin and Jones, 1961); lower genital lip (Spielman, 1964).

Lower Vaginal Sclerite (LVS) = A pigmented sclerite of the vaginal floor other than the lower vaginal lip, usually absent, however, well developed and usually spiculate in many species of *Aedes* subgenera *Neomacleaya* and *Verrallina*. Syn.: preatrial plate (Laffoon, 1946).

Peri-anal Membrane (PaM) = Membrane attached to ventrobasal portion of the cerci, extending around the anus and attached to the dorsal surface of the postgenital lobe, the latter point of attachment (DPGL) usually appears as an inverted "U"; with scattered spicules. Syn.: mamelon anal (Brolemann, 1919); tenth segment (Christophers, 1923); anal membrane or proctiger (Gerry, 1932); anal segment (Gjullin, 1937); anal cone or proctiger (Jones and Wheeler, 1965).

Postgenital Lobe (PGL) = A median ventral lobe extended ventrally below the anus, weakly to moderately sclerotized, setose and with or without an apical median indentation. Syn.: palmette sous-anale (Brolemann, 1919); ventral process of tenth segment (Macfie and Ingram, 1922); postgenital plate (Christophers, 1923); tenth sternite (in part) (Cerqueira, 1943); postpygidal plate (Curtin and Jones, 1961).

Segment VIII = Usually modified from preceding abdominal segments and more or less retractile; when retracted usually without or with only a very few scales.

Seminal Capsule (SCa) = Any one of the 1-3 pigmented, enlarged, usually spherical caeca at the inner end or ends of the spermathecal ducts, usually 1 seminal capsule larger than others. Syn.: spermatheca (most authors); spermatotheca (Hara, 1957).

Seminal Capsule Pore (SCaP) = A series of minute pores located near the orifice of the seminal capsule and appear as small, circular, clear spots in mounted specimens (pores larger and scattered over most of seminal capsule surface in anophelines). Syn.: pale spots (Macfie and Ingram, 1922); dots (La Casse and Yamaguti, 1950); transparent dots *(Anopheles)* (Hara, 1959).

Spermathecal Eminence (SE) = A median projection extending into the vagina from its roof and bearing the apertures of the spermathecal ducts and accessory gland duct; unpigmented in most genera and subgenera but strongly sclerotized and pigmented in *Aedes* subgenera *Neomacleaya* and *Verrallina*, species of the former subgenus also with spiny excrescences on cephalic margin. Syn.: median plate (Edwards, 1941); postatrial plate (in part) (Laffoon, 1946); post-atrial apparatus (?) (Mattingly, 1958); dorsal plate (Curtin and Jones, 1961).

Spiny Excrescences (Ex) = Outgrowths from the spermathecal eminence, vary from small hair-like spines to long, thick, branched structures; in the Aedini well developed in *Aedes* subgenera *Neomacleaya* and *Verrallina*. Syn.: postatrial hairs (Laffoon, 1946); postatrial plate hairs (Delfinado, 1967).

Sternum VIII (VIII-S) = Sclerite variable in shape, width usually broader than length, apex may have a median indentation, larger than tergum VIII, setose, scales present or absent. Syn.: viii sternite (Macfie and Ingram, 1922).

Sternum VIII Index = Ventral measurement of distance from apex to most anterior point of base (sternum VIII length) divided by measurement of width at widest point (sternum VIII width).

Tergum IX (IX-Te) = A small plate situated between apex of tergum VIII and base of cerci, variable in shape and pigmentation, setae present or absent. Syn.: ix tergite (Macfie and Ingram, 1922).

Tergum IX Index = Dorsal measurement of distance from apex to the most anterior point of base (tergum IX length) divided by measurement of width at widest point (tergum IX width).

Tergum VIII (VIII-Te) = Sclerite variable in shape, usually more or less trapezoidal, setose apically, scales present or absent. Syn.: viii tergite (Macfie and Ingram, 1922).

Tergum VIII Index = Dorsal measurement of distance from apex to most anterior point of base (tergum VIII length) divided by measurement of width at widest point (tergum VIII width).

Tuberculus (Tu) = One or more small, rounded, pale button-like structures situated on the insula, occasionally with a minute seta or spicule in the center.

Upper Vaginal Lip (UVL) = Sclerotized and pigmented rim of the roof of the vagina, cephalad area articulates with the lower vaginal lip at hinge, caudal margin joined to basoventral margin of postgenital lobe, usually spiculate. Syn.: bride dorsale (Brolemann, 1919); sigma (in part) + cowl (Christophers, 1923); ninth sternite (Gerry, 1932); cowl (Barraud, 1928); postatrial sclerite (Laffoon, 1946); posterior sigma + anterior cowl (Coher, 1948); dorsal arc of sigma (Snodgrass, 1959); upper genital lip (Spielman, 1964).

Upper Vaginal Sclerite (UVS) = A pigmented sclerite of the vaginal roof other than the upper vaginal lip, usually attached to inner cephalic area of lip, may be continuous with lip and distinguishable by position only, nonspiculate. Syn.: atrial plate (Christophers, 1923); postatrial plate (in part) (Laffoon, 1946).

Vagina (Va) = An ectodermal pouch derived from the venter of abdominal segment IX, pouch is partially divided transversely by spermathecal eminence and in a number of genera and subgenera by the upper vaginal sclerites. Syn.: genital orifice (Macfie and Ingram, 1922); atrium (Christophers, 1923).

Ventral Postgenital Lobe Index (Ventral PGL Index) = Ventral measurement of distance from apex to midpoint of posterior margin of the upper vaginal lip (ventral PGL length) divided by measurement of width at 0.5 of dorsal length (dorsal PGL width).

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MORPHOLOGY OF FEMALE GENITALIA

Fig. 1